Optimization of Ggene Expression and Purification of Legionella Pneumophila Peptidoglycan Associated Lipoprotein (PAL) Recombinant Protein

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Background & Objectives: Legionellae including *Legionella pneumophila*, are recognized as the causative agents for community, travel and hospital-acquired pneumonia. There are many problems with most of available diagnostic tests used to diagnose *Legionella pneumonia*, including inadequate sensitivity and specificity, and inability to provide a result in a clinically useful time period but among them, the urinary antigen tests have revolutionaized the laboratory diagnosis of *Legionella pneumonia*. Since the peptidoglycan-associated lipoprotein (PAL) protein of *L. pneumophila* is an urinary antigen and considered as useful diagnostic antigen to diagnose Legionella infection, the aim of this study was to optimaize expression and purification of *L. pneumophila* PAL protein.

Methods: In this experimental study, optimizing of 5 parameters (cell density, induction time, growth temperature, IPTG concentration and type of medium) was performed. After expression, periplasmic extract was prepared and recombinant PAL protein purified using Ni2+-charged resin column. Finally, recombinant PAL protein was verified by Western blotting.

Results: The optimum expression of the r-PAL protein was occurred at an OD600 of 0.6, 1mM IPTG concentration, after 15 hours at 25ºC and use of Terrific Broth medium. Recombinant periplasmic PAL protein was highly purified (>80%) using Ni-NTA column. Also, western blotting analysis showed that recombinant PAL protein was specifically recognized by anti-His6-peroxidase antibody.

Conclusion: In order to increase the functional expression of recombinant proteins, a number of strategies were employed such as optimization of expression conditions and periplasmic expression. Also, the results obtained from this study supported the idea that *E. coli* BL21

strain can be served as a functional host for pET26b vector and the designed host and vector can be used in large scale production of recombinant PAL protein

Keywords: Optimization; Expression; Purification; Peptidoglycan Associated Lipoprotein (PAL); *Legionella Pneumophila*